# Ni CHELATION: ICP METHOD

## Why to do this :

1. To determin the quantity of curli

## What you need :

1. Culture media : LB

- 10 g bactotrypton
- 5 g yeast extract
- 5 g NaCl
- 0,5 mL NaOH 10N
- Qsp 1 L

2. Antibiotics concentrations

Chloramphenicol (Cm) : 2 mg/mL
Tetracycline (Tet) : 1 mg/mL
Kanamycin (Kann) : 5 mg/mL
Ampicillin (Amp) : 10 mg/mL

→ 50 µL antibiotic / 5mL medium

3. Apparatus : Agilent 7500cx

## How to do :

1. Bacteria culture

a) Prepare 50mL of liquid culture
b) Take 20mL of this culture (if you are testing several strains at once, adjust the quantity in order to have the same OD for each strain)

2. Preparing the bacteria

a) Centrifuge 10 min at 10000g
b) Add 5mL of ultrapure water
c) Remove supernatant
d) Centrifuge 10 min at 10000g
e) Remove the water (be careful not to dissolve the pellet, do this step one more time if needed)
f) Add 20mL of Nickel solution at 100uM
g) Leave them in contact for 30min, on the rotating.
h) Centrifuge 10 min at 10000g
i) Remove the non-colored supernatant, centrifuge again if needed until the supernatant is totally removed
j) Keep the pellet
k) Measure the OD at 600nm of the supernatant for each strain.

3. Step 2bis

a) The pullet (from 5 to 15 mg of body weight material) is then mineralized with (1,5ml) nitric acid 69% at 105°C during 3 hours

b) The mineralization tubes are filled with deionized water to 50 ml

c) Prior to ICP analysis, sample are diluted from 100 to 10000 times with a mixture HNO3 0.5% / In 2% (Indium is used as an internal standard in order to have any signal variation)

d) ICP-MS analysis were carried out with a calibration curve going from 1 ppb to 100 ppb (1 to 100 µg/L)